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liver lysate is made for use as a positive control. Finally, 45 μ l of solubilized tissue or diluted sense strand is mixed directly with either: (1) 1×10^5 cpm of radioactively labeled probe; or (2) 250 pg of non-isotopically labeled probe in 5 μ l of lysis buffer. Hybridization is allowed to proceed overnight at 37°C. See, T. Kaabache et al., Anal. Biochem. 232:225-230 (1995).

C. RNase Digestion. RNA that is not hybridized to probe is removed from the reaction as per the DIRECT PROTECT™ protocol using a solution of RNase A and RNase T1 for 30 min at 37°C, followed by removal of RNase by Proteinase K digestion in the presence of sodium sarcosyl. Hybridized fragments protected from digestion are then precipitated by the addition of an equal volume of isopropanol and placed at -70°C for 3 hr. The precipitates are collected by centrifugation at 12,000 x g for 20 min."

Please amend page 69, lines 28-33 as follows:

EB
"Many other detection formats exist which can be used and/or modified by those skilled in the art to detect the presence of amplified or non-amplified LS 147-derived nucleic acid sequences including, but not limited to, ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL); Q-beta replicase (GENE-TRAK™, Naperville, Illinois), branched chain reaction (Chiron, Emeryville, CA) and strand displacement assays (Becton Dickinson, Research Triangle Park, NC)."

Please amend page 74, lines 21-36 and page 75, lines 1-14 as follows:

EF
"A plasmid for the expression of secretable LS147 proteins is constructed by inserting an LS147 polynucleotide sequence from clone 1362407 into the pcDNA3.1/Myc-His vector. (This plasmid will be referred to as pc1362407-M/H.) Prior to construction of pc1362407-M/H, the LS147 cDNA sequence is first cloned into a pCR®-Blunt vector as follows: The LS147 cDNA fragment is generated by PCR using standard procedures using reagents from STRATAGENE®, Inc. (La Jolla, CA) as directed by the manufacturer. PCR primers are used at a final concentration of 0.5 μ M. PCR using 5 U of pfu polymerase (STRATAGENE®, La Jolla, CA) is performed on the

LS147 plasmid template (see Example 2) in a 50 µl reaction for 30 cycles (94°C, 1 min; 65°C, 1.5 min; 72°C, 3 min) followed by an extension cycle of 72°C for 10 min. The sense PCR primer sequence is identical to that found directly upstream of the LS147 insertion site in the pINCY vector. The antisense PCR primer sequence incorporates a 5' NotI restriction sequence and a sequence complementary to the 3' end of the LS147 cDNA directly upstream of the 3'-most in-frame stop codon. Five microliters (5 µl) of the resulting blunted-ended PCR product are ligated into 25 ng of linearized pCR®-Blunt vector (Invitrogen, Carlsbad, CA) interrupting the lethal ccdB gene of the vector. The resulting ligated vector is transformed into TOP10 E. coli (Invitrogen, Carlsbad, CA) using a ONE SHOT™ transformation kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The transformed cells are grown on LB-Kan (50 µg/ml kanamycin) selection plates at 37°C. Only cells containing a plasmid with an interrupted ccdB gene will grow after transformation [Grant, S.G.N., PNAS 87:4645-4649 (1990)]. Transformed colonies are picked and grown up in 3 ml of LB-Kan broth at 37°C. Plasmid DNA is isolated by using a QIAPREP® (Qiagen Inc., Santa Clarita, CA) procedure, as directed by the manufacturer. The DNA is digested with EcoRI or SnaBI, and NotI restriction enzymes to release the LS147 insert fragment. The fragment is electrophoresed on a 1% SEAKEM® LE agarose/0.5 µg/ml ethidium bromide/TE gel, visualized by UV irradiation, excised and purified using QIAQUICK™ (Qiagen Inc., Santa Clarita, CA) procedures, as directed by the manufacturer."

Please amend page 75, lines 31-36 and page 76, lines 1-19 as follows:

"B. Transfection of Human Embryonic Kidney Cell 293 Cells. The LS147 expression plasmid described in section A, supra, is retransformed into DH5α™ cells, plated onto LB/ampicillin agar, and grown up in 10 ml of LB/ampicillin broth, as described hereinabove. The plasmid is purified using a QIAFILTER™ Maxi kit (Qiagen, Chatsworth, CA) and is transfected into HEK293 cells (F.L. Graham et al., J. Gen. Vir. 36:59-72 (1977)). These cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, MD 20852, under Accession No. CRL 1573. Transfection is carried out using

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the cationic lipofectamine-mediated procedure described by P. Hawley-Nelson et al., Focus 15.73 (1993). Particularly, HEK293 cells are cultured in 10 ml DMEM media supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM) and freshly seeded into 100 mm culture plates at a density of 6.5×10^6 cells per plate. The cells are grown at 37°C to a confluency of between 70% and 80% for transfection. Eight micrograms (8 µg) of plasmid DNA are added to 800 µl of Opti-MEM I® medium (Gibco-BRL, Grand Island, NY), and 48-96 µl of LIPOFECTAMINE™ Reagent (Gibco-BRL, Grand Island, NY) are added to a second 800 µl portion of Opti-MEM I® media. The two solutions are mixed and incubated at room temperature for 15-30 min. After the culture medium is removed from the cells, the cells are washed once with 10 ml of serum-free DMEM. The Opti-MEM I- LIPOFECTAMINE™-plasmid DNA solution is diluted with 6.4 ml of serum-free DMEM and then overlaid onto the cells. The cells are incubated for 5 hr at 37°C, after which time, an additional 8 ml of DMEM with 10% FBS are added. After 18-24 hr, the old medium is aspirated, and the cells are overlaid with 5 ml of fresh DMEM with 5% FBS. Supernatants and cell extracts are analyzed for LS147 gene activity 72 hr after transfection.”

Please amend page 77, lines 29-35 as follows:

EG
“E. Coating Microtiter Plates with LS147 Expressed Proteins. Supernatant from a 100 mm plate, as described supra, is diluted in an appropriate volume of PBS. Then, 100 µl of the resulting mixture are placed into each well of a REACTI-BIND™ metal chelate microtiter plate (Pierce, Rockford, IL), incubated at room temperature while shaking, and then is washed four times with deionized water. The prepared microtiter plate can then be used to screen polyclonal antisera for the presence of LS147 antibodies (see Example 17).”

REMARKS

Reconsideration of the above-identified application is respectfully requested.